

Host-parasite associations in a population of the nectarivorous bat *Anoura geoffroyi* (Phyllostomidae) in a cave in a Brazilian ferruginous geosystem

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Abstract

Parasitic relationships between Neotropical bats and their ectoparasites are not well known, even though parasitism is one of the factors that can affect the fitness of a host population. This study characterized parasite-host relationships in relation to sex, age, body size and reproductive status in a population of *Anoura geoffroyi* using the indices of Prevalence, Mean Intensity and Mean Abundance. Total prevalence for 93 sampled bats was 94.6%. Two species of streblid flies that are considered primary parasites of *A. geoffroyi*, *Exastinion clovisi* (n = 203) and *Anastrebla modestini* (n = 152), were the most abundant ectoparasites, followed by *Trichobius* sp. (n = 7). Two mite species, *Periglischrus vargasi* (Spinturnicidae) (n = 98) and *Spelaeorhynchus praecursor* (Spelaeorhynchidae) (n = 11), were also found. We recorded higher mean abundance and intensity of parasitism in pregnant females compared to reproductive males and reproductively inactive females, for different specific associations of ectoparasites. Host age and body condition had no effect on the parasitological indices. Even with high rates of parasitism, parasitic load did not influence host body condition, but infestation rates by mites were higher in reproductive males and higher by flies in reproductive females, showing that ectoparasites can have variable influences between the different stages of the life history of these host bats. Thus, the reproductive activity of the hosts could be an adverse factor for resistance to parasite infestations.

Keywords

Body Condition Index, Ectoparasitic relationships, Parasitological indexes

Introduction

Ectoparasitism can have a strong influence on host populations (Brown and Brown 2004; Møller and Saino 2004). Its diversity is directly influenced by several factors such as geographic distribution, roost environment and by host biology, morphology and behavior (Rui and Graciolli 2005; Postawa and Nagy 2016). Pressures resulting from parasitism can increase the rate of predation on hosts, weaken host physical condition, and increase disease incidence, which can result in decreases in survival and reproduction (Neuhaus 2003; Brown and Brown 2004; Ter Hofstede and Fenton 2005). Ectoparasites may have more significant influence on bat populations, as most bats are gregarious, that can form colonies of thousands of individuals and often roost in places with limited space, such as caves (Bredt et al. 1999; Lourenço and Palmeirim 2007, 2008; Guimarães and Ferreira 2014), which is the case for *Anoura geoffroyi* Gray, 1838 (Guimarães and Ferreira 2014; Farias et al. 2018; Reis 2018).

Anoura geoffroyi (Chiroptera: Phyllostomidae) is a nectarivorous bat (13–18 g) (Koopman 1994; Reid 1997) with a wide geographic distribution in the Neotropical region, from Mexico to Peru, Bolivia, and Brazil (Simmons 2005). The species has a strong association with natural cavities and preferably uses caves as diurnal roosts (Arita 1993; Guimarães and Ferreira 2014), where colonies of hundreds of individuals can form (Bredt et al. 1999; Farias et al. 2018; Reis 2018). The species presents seasonal population dynamics with a monoestrous reproductive pattern and reproductive activity occurring during the rainy season (Zortéa 2003; Farias et al. 2018).

Among the species of ectoparasites found on bats dipterans and mites are the most common. Dipterans of the family Streblidae are obligatory blood-sucking ectoparasites of bats, they are viviparous and have three larval stages that develop in the female's uterus, the pupa that develops in the host's roost and the adult, which is the hematophagous ectoparasite (Dick and Patterson 2006). Likewise, mites of the family Spinturnicidae are exclusive parasites of bats and complete their entire life cycle on the host's body. The dispersion of these mites requires direct contact between hosts, which is facilitated by the social behaviors of bats, such as copulation, birthing of young, parental care and the habit of living in groups. They are commonly found on the patagium of the host (Rudnick 1960; Lourenço and Palmeirim 2007; Almeida et al. 2015). In temperate region, it was observed that sex, age, and reproductive status of the host strongly influences the reproductive activity of parasites, and, according to the authors, reproduction of ectoparasites of many temperate cave-dwelling bats is mostly regulated by the reproductive cycle of their bat hosts (Lourenço and Palmeirim 2008).

Studies related to different aspects of the association between ectoparasites and bats in the Neotropical region are still scarce. Thus, there is a knowledge gap about parasitological relationships resulting from the numerous factors that influence ectoparasitism in bats such as sex, age and reproductive status of the host, among others (Rui and

Graciolli 2005; Patterson et al. 2007; Patterson et al. 2008; Postawa and Nagy 2016). The present study aimed to understand the ecological aspects involved in the interaction between ectoparasites and the phyllostomid bat *A. geoffroyi* in a colony that uses a ferruginous cave as a diurnal roost and has been present at the site for many years. The effects of bat sex, age, reproductive status, and body condition on the rate of ectoparasite infestation were investigated. Considering that the species exhibits seasonal monoestric reproductive activity during the rainy season, we hypothesize here that ectoparasites may have variable influences between the different stages of the hosts' life history, as well as in relation to their body condition.

Methods

Study area

The studied colony of *A. geoffroyi* lives in the ferruginous cave named Piedade (19°49'20"S, 43°40'33"W, 1,414 m altitude). The colony is formed by groups of varying sizes (5 to 20 individuals). Maximum abundance is observed in the reproductive period with a few hundred of individuals (Farias et al. 2018). The cave is situated in Serra da Piedade, located in Monumento Natural Estadual Serra da Piedade (state park), state of Minas Gerais, Brazil, and has a horizontal projection of about 360 m (Pereira et al. 2012). Serra da Piedade is situated in the Quadrilátero Ferrífero ferruginous geosystem (Bueno 1992), which harbors the largest iron ore reserve in Brazil (Souza and Carmo 2015). At present, there are 46 open pit mines reported from the region, which may pose a threat to local fauna and flora (Souza and Carmo 2015). The vegetation in the region varies with altitude, with semi-deciduous forest in the lower parts and altitudinal fields or rupestrian field at higher altitudes (Bueno 1992). The climate of the region is subtropical of altitude (Cwa), according to the Köppen classification. There are two well defined seasons with a rainy season from October to March (corresponding to spring-summer months) and a dry season from April to September (corresponding to the autumn-winter months) (Bueno 1992).

Data collection

Diurnal campaigns to the roost of *A. geoffroyi* (cave environment) were carried out on September 9, 2017; January 24, 2018; and September 18, 2018 to capture bats and collect ectoparasites. Bats were captured with a mist-net (12 × 3 m) installed inside the cave about 50 m from the colony, from 8:00 h to 14:00 h, which was checked every 20 minutes. To minimize the disturbance of the colony, the researchers remained outside waiting for the capture of bats in flight. With at least three openings inaccessible, the placement of mist nets outside the cave was not possible. The use of a pole net was also not possible due to the great height of the cave. Due to the difficulties imposed by local conditions, diurnal collections were used, which proved to be viable following the protocol of Farias et al. (2018). Information related to sex, age, body mass (grams), forearm

length (millimeters) and reproductive status was obtained for each captured animal. Bats were released without marking, shortly after the collection of ectoparasites and biometric data. The degree of ossification of the metacarpal epiphyses was evaluated to determine age (Anthony 1988). Reproductive status was analyzed by observing secondary reproductive characteristics (Farias et al. 2018). Inactive males had poorly developed testes, while active males presented fully developed scrotal testes. Pregnant or lactating females were considered reproductively active, while others were considered inactive.

Ectoparasites were collected by inspecting the pelage of the bats with the naked eye and using fine-tipped forceps to transfer them to individual containers (containing 70% ethanol) for each bat (Graciolli and Carvalho 2001). The ectoparasites were prepared as described in DeBlase and Martin (1980). Samples of each ectoparasite species were chosen under a stereomicroscope, and then these samples were placed on a microscope slide and cleared in Hertwig's solution (chloral hydrate). This solution is a clearing agent for microscopic examination and is commonly used in the diet analysis of small mammals (DeBlase and Martin 1980; Talamoni et al. 2008). After this process, the ectoparasites were mounted on a slide in a drop of glycerin and covered by a coverslip. Dipterans and mites were then identified under an optical microscope (10×), using dichotomous keys (Graciolli and Carvalho 2001; Herrin and Tipton 1975; Wenzel et al. 1976; Peracchi 1990).

Parasitological indices were calculated to analyze the infestation in the population and the association with each parasite, except for the species *Spelaeorhynchus praecursor* Neuman, 1902 (Spelaeorhynchidae), which was poorly sampled in the present study. Prevalence (P; number of infested hosts/number of hosts examined \times 100) expressed as a percentage, Mean Intensity (MI; number of parasites/number of infested hosts) and Mean Abundance (MA; number of parasites/total number of hosts examined) were calculated (Bush et al. 1997). The influence that host sex, age (adult, non-adult) and reproductive status have on the parasitological indexes was investigated. All young and sub-adult animals of both sexes were included in the non-adult class, being differentiated by the presence of cartilaginous epiphyses and all being sexually immature (Farias et al. 2018). Mean intensities and mean abundances were compared by Student's *t*-test with randomization and 2000 replicates. The influence of host biological parameters on prevalence was evaluated by Fisher's exact test. Analyses were performed using Quantitative Parasitology 3.0 software (Rózsa et al. 2000). The Body Condition Index (BCI) (body mass/forearm length; Reichard and Kunz 2009) was used to investigate the relationship between host body condition and parasitic load through Spearman correlation tests. All tests were run using Bioestat 5.3 software (Ayres et al. 2007) with a significance level of 5%.

Results

A total of 93 bats were captured, 88 of which were infested, resulting in a prevalence rate of 94.6% (0.8–1.0, CI 95%). The total of 471 ectoparasites collected included flies of three species of the family Streblidae – *Anastrebla modestini* Wenzel, 1966

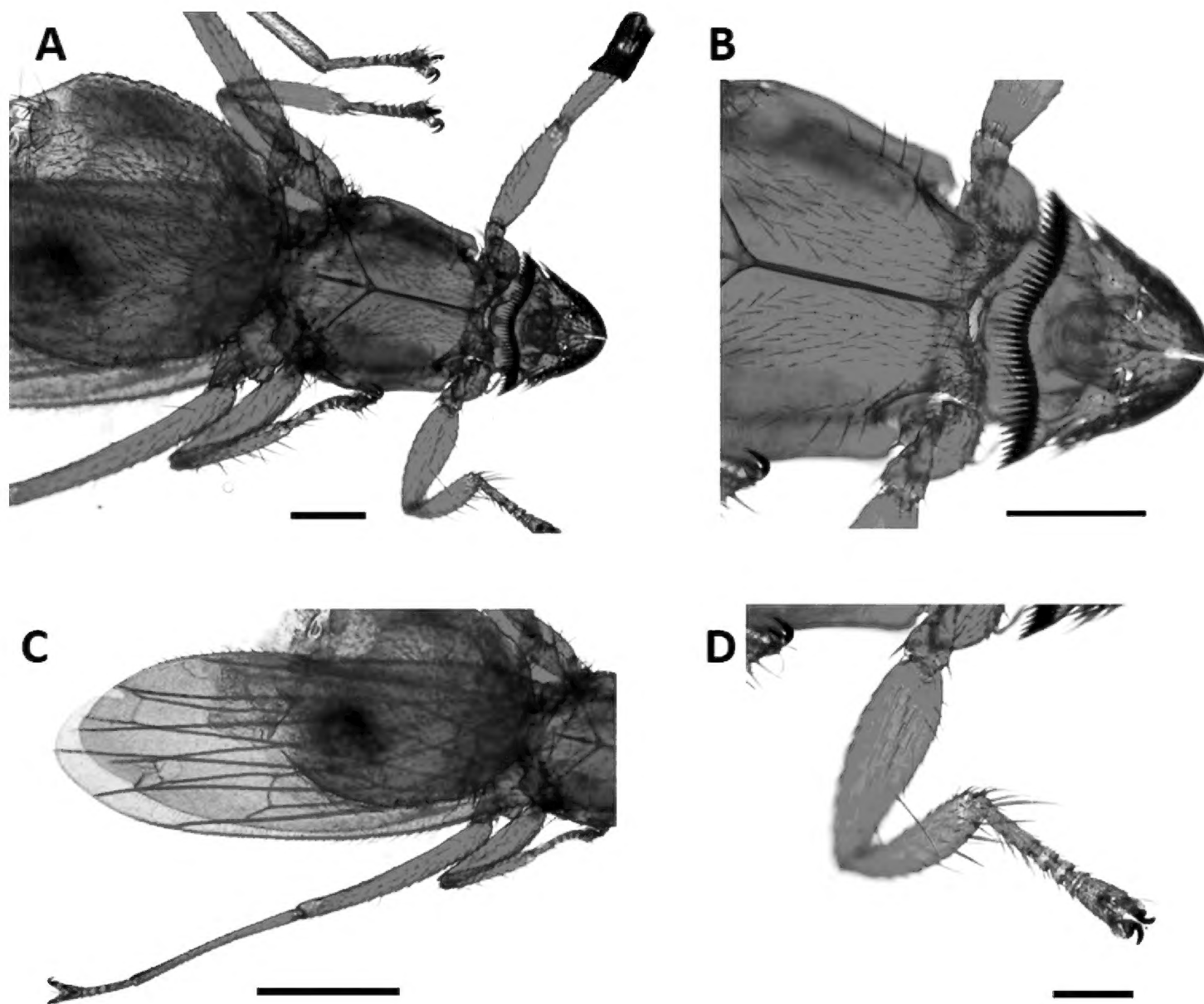


Figure 1. **A** ventral view of *Anastrebla modestini*, 40× magnification **B** detail of head, 100× **C** detail of wing and leg, 100× **D** detail of femur and setae, 100×. Leica DM500 optical microscope. Scale bars: 0.5 mm (**A–D**).

($n = 152$, Fig. 1), *Exastinion clovisi* (Pessoa & Guimarães, 1936) ($n = 203$, Fig. 2) and *Trichobius* sp. ($n = 7$, Fig. 3). Specimens of two species of mites were also collected – *Periglischrus vargasi* Hoffmann, 1944 (Spinturnicidae, $n = 98$, Fig. 4) and *S. praecursor* ($n = 11$, Fig. 5). Mean Intensity (MI) was $5.35 (\pm 3.47)$ ectoparasites per host while Mean Abundance (MA) was $5.06 (\pm 3.58)$ ectoparasites per host.

Infestation analysis for all ectoparasites (Table 1) revealed that host sex did not affect P ($p = 1.00$), MA ($t = 1.14$; $p = 0.24$) or MI ($t = 1.31$; $p = 0.18$). Reproductively active and inactive males showed no differences in the indexes (P: $p = 1.0$; MI: $t = -1.31$, $p = 0.21$; MA: $t = -1.52$, $p = 0.16$), as was also the case for pregnant and inactive females (P: $p = 0.55$; MI: $t = 0.30$, $p = 0.75$; MA: $t = 0.83$, $p = 0.39$). On the other hand, pregnant females had higher MA than reproductively active males ($t = 2.12$, $p = 0.03$) (Table 1).

For the specific association between *A. geoffroyi* and *E. clovisi* (Table 2), female hosts had higher MA ($t = 2.31$, $p = 0.02$) than did male hosts, while for the association between *A. geoffroyi* and *A. modestini* (Table 2), pregnant females had higher P ($p = 0.02$) and higher MA ($t = 2.87$, $p = 0.006$) than did inactive females. Pregnant

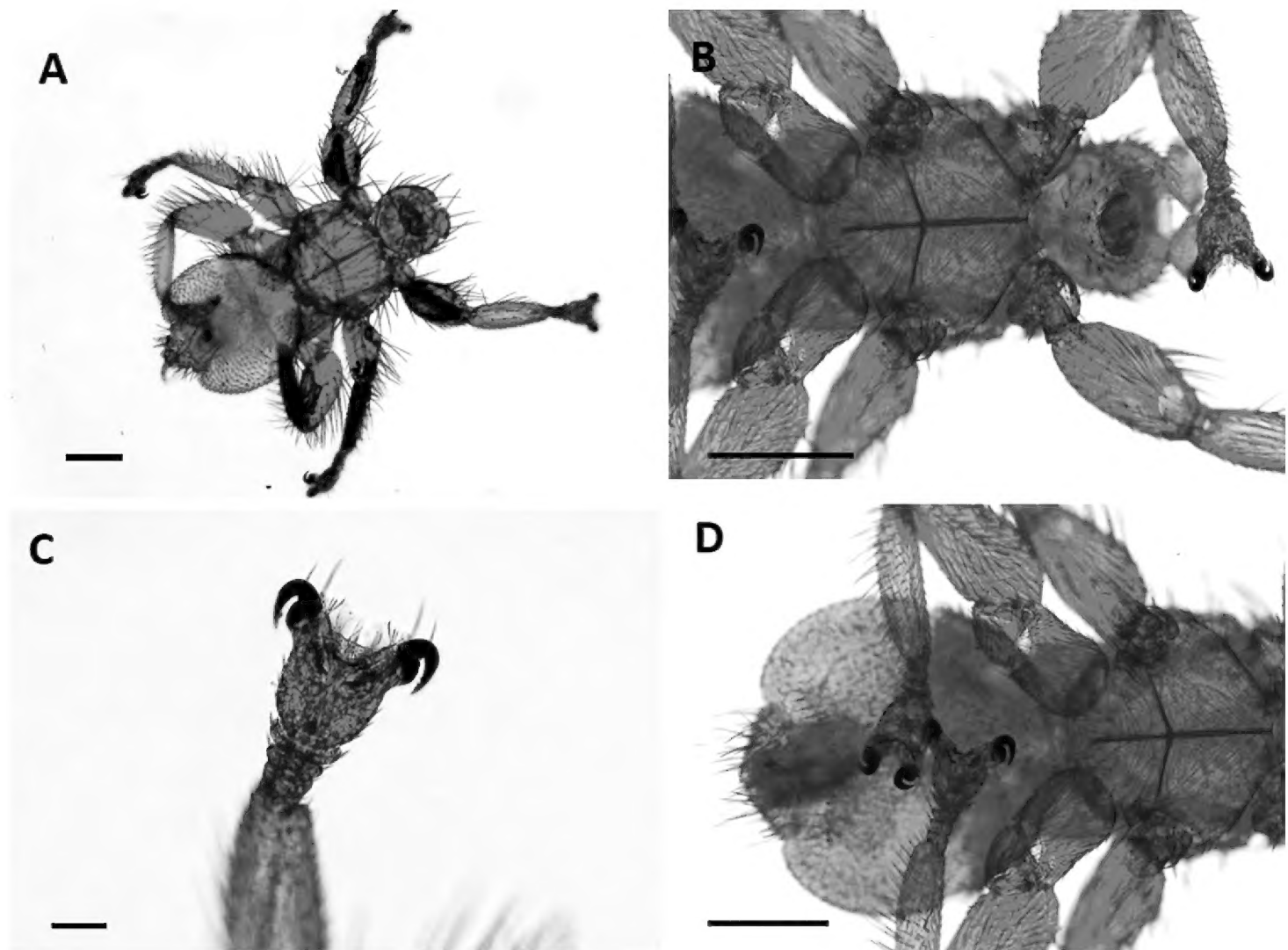


Figure 2. **A** dorsal view of *Exastinion clovisi*, 40× magnification **B** detail of thorax, 100× **C** distal detail of the leg, 100× **D** detail of abdomen, 100×. Leica DM500 optical microscope. Scale bars: 0.5 mm (**A**, **B**, **D**); 0.1 mm (**C**).

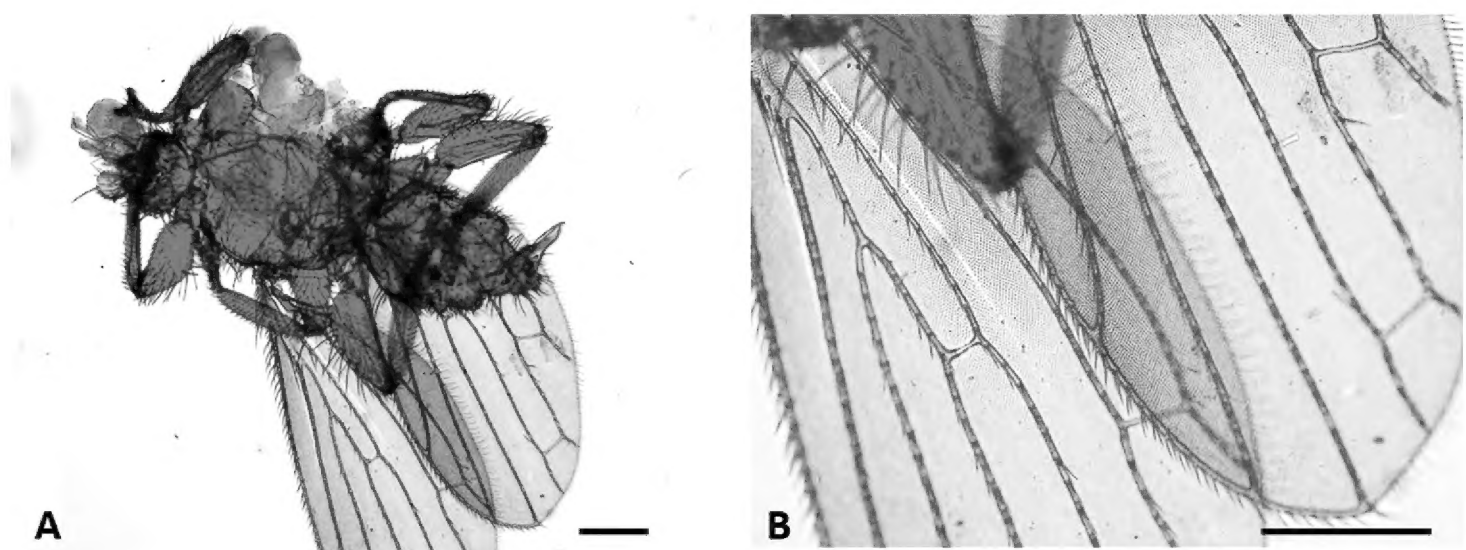


Figure 3. **A** dorsal view of *Trichobius* sp., 40× magnification **B** detail of wing, 100×. Leica DM500 optical microscope. Scale bars: 0.5 mm (**A**, **B**).

females were also more parasitized than reproductive active males, with significant differences in P ($p = 0.005$), MA ($t = 3.32$, $p = 0.006$) and MI ($t = 1.99$, $p = 0.04$). For the association between *A. geoffroyi* and the mite *P. vargasi* (Table 2), MA and MI were

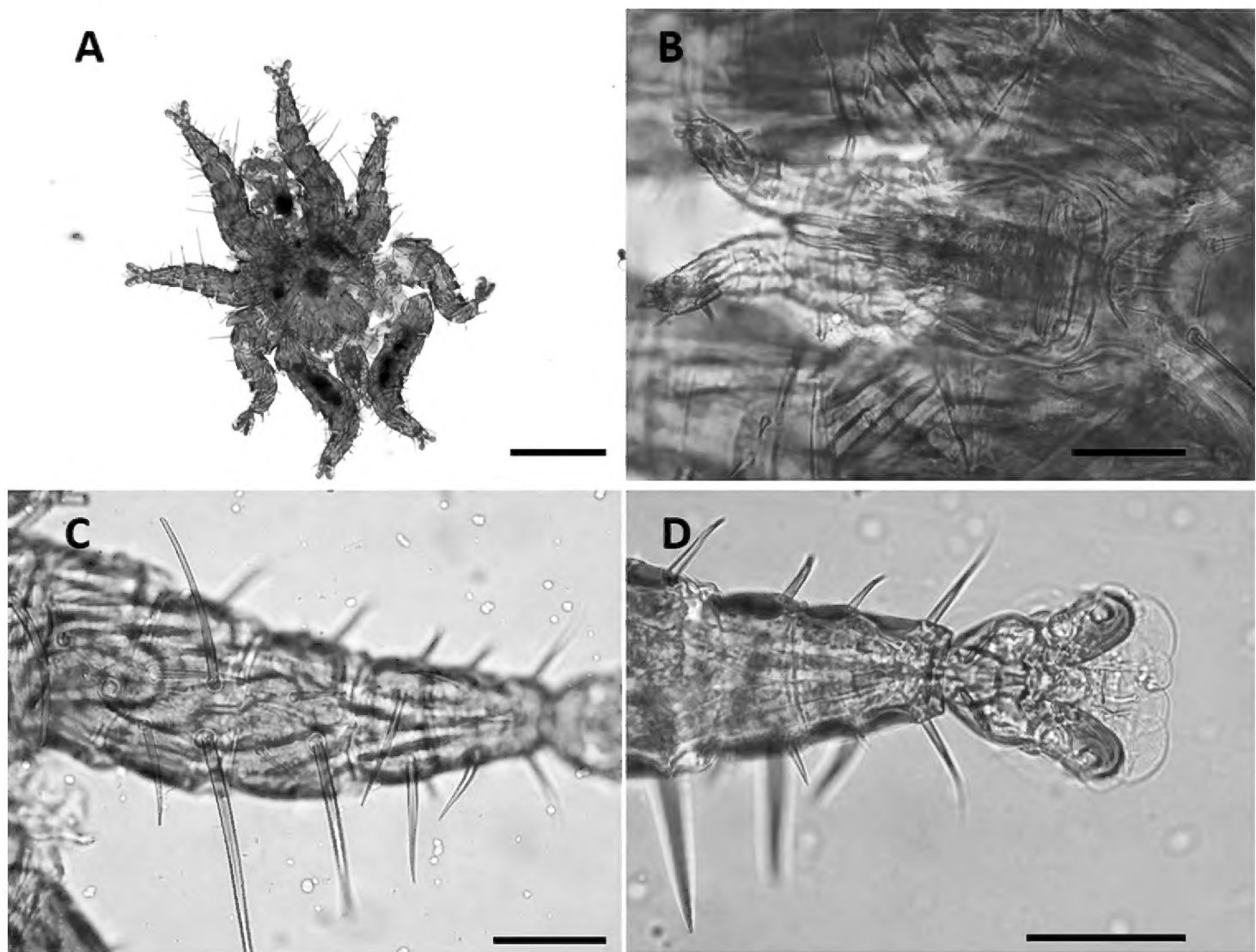


Figure 4. **A** specimen of *Periglischrus vargasi*, 40× magnification **B** detail of gnathosome, 400× **C** detail of setae insertion in the leg, 400× **D** distal detail of the leg, 400×. Leica DM500 optical microscope. Scale bars: 0.5 mm (**A**); 0.1 mm (**B–D**).

Table 1. Number of hosts examined (N), infected (in parentheses), Prevalence (P), Mean Intensity (MI) and Mean Abundance (MA), with Confidence Intervals (CI 95%), for ectoparasites of *Anoura geoffroyi* in Piedade cave, located in Serra da Piedade, state of Minas Gerais. Inactive males had poorly developed testes and inactive females were those who did not show evidence of pregnancy and lactation. * = Significant differences.

Host		N	P (%) (CI 95%)	MI (CI 95%)	MA (CI 95%)
Sex	Female	50 (47)	94.0 (0.8–0.9)	5.8 (4.8–6.8)	5.4 (4.4–6.4)
	Male	43 (41)	95.3 (0.8–0.9)	4.8 (3.8–6.0)	4.6 (3.6–5.7)
Reproductive status	Reproductive male	32 (30)	93.8 (0.7–0.9)	4.2 (3.3–5.4)	4.0 (3.0–5.0)*
	Inactive male	11 (11)	100 (0.7–1.0)	6.3 (3.9–9.3)	6.3 (3.9–9.3)
	Pregnant female	18 (18)	100 (0.8–1.0)	6.0 (4.5–7.5)	6.0 (4.5–7.5)*
	Inactive female	32 (29)	90.6 (0.7–0.9)	5.6 (4.5–6.9)	5.1 (3.9–6.4)
Age	Adult	52 (51)	98.1 (0.8–0.9)	5.1 (4.3–6.0)	5.0 (4.2–5.9)
	Non-adult	41 (37)	90.2 (0.7–0.9)	5.6 (4.5–6.9)	5.0 (3.9–6.4)

higher for pregnant females compared to inactive females (MA: $t = -2.79$, $p = 0.009$; MI $t = -2.93$, $p = 0.01$), while reproductively active males were more parasitized than pregnant females with respect to MA and MI (MA: $t = -2.34$, $p = 0.02$; MI: $t = -3.17$, $p = 0.01$) (Table 2).

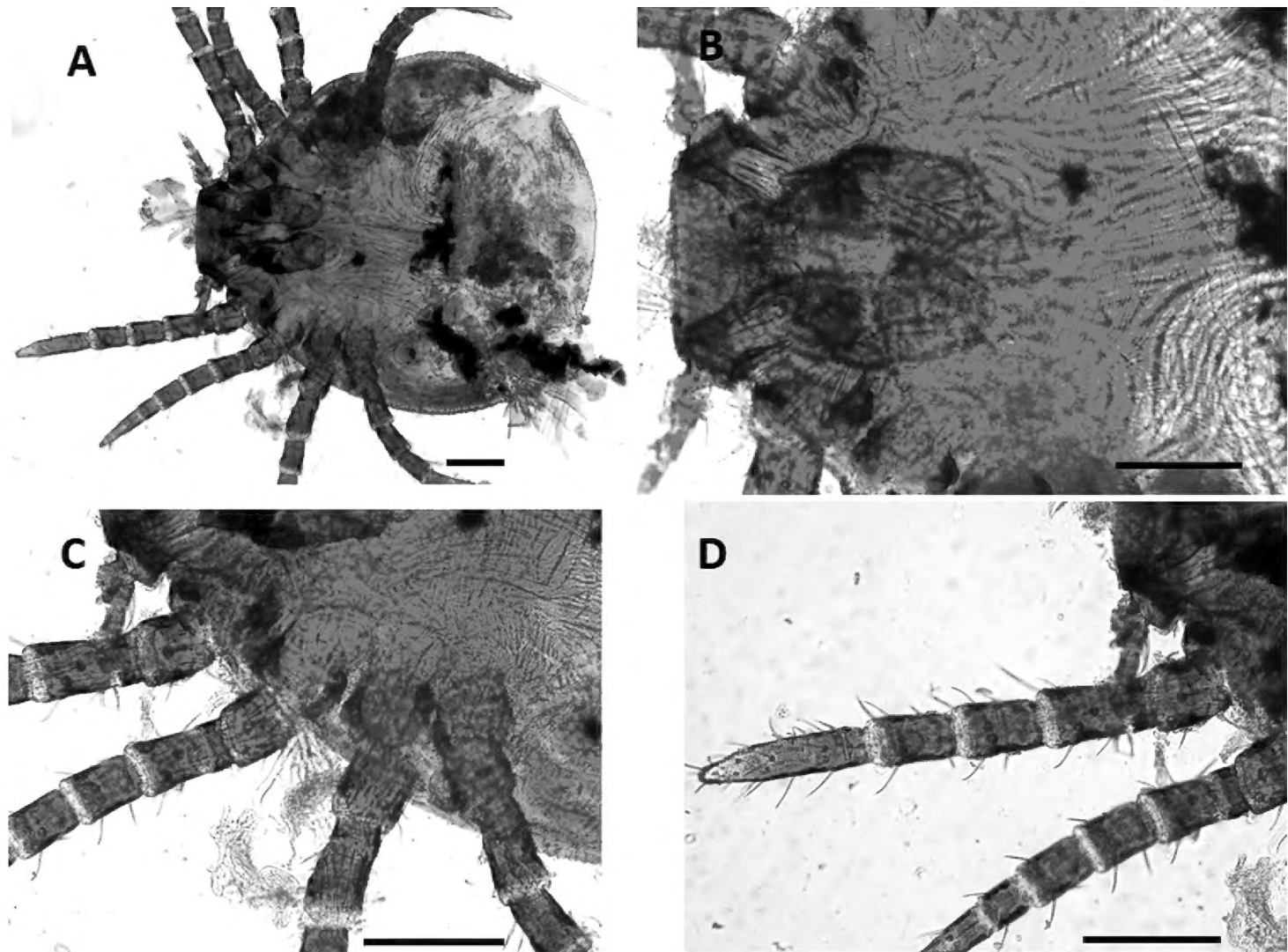


Figure 5. **A** specimen of *Spelaeorhynchus praecursor*, 40× magnification **B** detail of gnathosome, 100× **C** detail of legs, 100× **D** detail of leg and setae, 100×. Leica DM500 optical microscope. Scale bars: 0.5 mm (**A–D**).

Analyses found no correlation between host BCI and parasitic load for males in general ($r_s = -0.05$; $n = 43$; $p = 0.70$); for reproductively active males ($r_s = -0.05$; $n = 32$; $p = 0.75$); for inactive males ($r_s = 0.19$; $n = 11$; $p = 0.75$); for females in general ($r_s = 0.002$; $n = 50$; $p = 0.98$); for pregnant females ($r_s = 0.17$; $n = 18$; $p = 0.740$); for inactive females ($r_s = -0.26$; $n = 32$; $p = 0.14$); for adults ($r_s = 0.13$; $n = 52$; $p = 0.33$) and for non-adults ($r_s = -0.21$; $n = 41$; $p = 0.17$).

Discussion

The present study registered two common species of parasitic flies belonging to the family Streblidae, *A. modestini* and *E. clovisi*, both having already been registered as primary parasites of *A. geoffroyi* (Wenzel et al. 1976). Both species are widely distributed in the Neotropical region, as are their hosts (Komeno and Linhares 1999; Graciolli and Rui 2001; Bertola et al. 2005; Simmons 2005; Moras et al. 2013; Dornelles and Graciolli 2017; Trujillo-Pahua and Ibáñez-Bernal 2018). *Trichobius* spp. was not abundant in the present study, although some species of the genus have been previously registered on *A. geoffroyi*, such as *Trichobius tiptoni* (Graciolli and Rui 2001) and *T. propinquus* (De Vasconcelos et al. 2015), or *Trichobius* sp. (*dugesii* complex) (Reis 2018).

Table 2. Number of hosts examined (N), infected (in parentheses), Prevalence (P), Mean Intensity (MI) and Mean Abundance (MA), with Confidence Intervals (95% CI), for specific interactions of *Exastinion clovisi*, *Anastrebla modestini* and *Periglischrus vargasi* with *Anoura geoffroyi* in Piedade cave, located in Serra da Piedade, state of Minas Gerais. Inactive males had poorly developed testes and inactive females were those who did not show evidence of pregnancy and lactation. * = Significant differences.

Host-parasite relationship		N	P (%) (CI 95%)	MI (CI 95%)	MA (CI 95%)
<i>Exastinion clovisi</i>					
Sex	Female	50 (40)	80.0 (0.6–0.8)	3.3 (2.7–4.1)	2.6 (2.0–3.4)*
	Male	43 (27)	62.0 (0.4–0.7)	2.5 (2.0–3.2)	1.6 (1.1–2.1)*
Reproductive status	Reproductive male	32 (18)	56.3 (0.3–0.7)	2.5 (1.9–3.1)	1.4 (0.9–2.0)
	Inactive male	11 (9)	81.8 (0.4–0.9)	2.6 (1.6–4.1)	2.1 (1.0–3.5)
	Pregnant female	18 (15)	83.3 (0.5–0.9)	2.9 (2.0–3.9)	2.4 (1.6–3.3)
	Inactive female	32 (25)	78.1 (0.6–0.9)	3.5 (2.6–4.6)	2.7 (1.9–3.7)
Age	Adult	52 (39)	75.0 (0.6–0.8)	2.9 (2.3–3.5)	2.1 (1.6–2.7)
	Non-adult	41 (28)	68.3 (0.5–0.8)	3.2 (2.4–4.1)	2.2 (1.5–3.0)
<i>Anastrebla modestini</i>					
Sex	Female	50 (33)	66.0 (0.5–0.7)	2.73 (2.1–3.4)	1.80 (1.2–2.4)
	Male	43 (26)	60.5 (0.4–0.7)	2.38 (1.6–3.8)	1.44 (0.9–2.4)
Reproductive status	Reproductive male	32 (18)	56.3 (0.3–0.7)*	1.89 (1.3–2.5)*	1.06 (0.6–1.5)*
	Inactive male	11 (8)	72.7 (0.3–0.9)	3.50 (1.5–7.5)	2.55 (1.0–6.0)
	Pregnant female	18 (17)	94.4 (0.7–0.9)*	3.06 (2.1–4.0)*	2.89 (2.0–3.7)*
	Inactive female	32 (16)	50.0 (0.3–0.6)*	2.38 (1.6–3.6)	1.19 (0.6–1.9)*
Age	Adult	32 (16)	67.3 (0.5–0.7)	2.60 (2.0–3.1)	1.75 (1.2–2.3)
	Non-adult	11 (8)	58.5 (0.4–0.7)	2.54 (1.7–4.0)	1.49 (0.9–2.5)
<i>Periglischrus vargasi</i>					
Sex	Female	50 (24)	48.0 (0.3–0.6)	1.7 (1.3–2.0)	0.84 (0.5–1.1)
	Male	43 (22)	51.2 (0.3–0.6)	2.5 (1.8–3.3)	1.30 (0.8–1.9)
Reproductive status	Reproductive male	32 (14)	43.8 (0.2–0.6)	2.7 (2.0–3.7)*	1.22 (0.6–1.9)*
	Inactive male	11 (8)	72.7 (0.3–0.9)	2.1 (1.2–3.6)	1.5 (0.7–2.8)
	Pregnant female	18 (6)	33.3 (0.1–0.5)	1.1 (1.0–1.3)*	0.3 (0.1–0.6)*
	Inactive female	32 (18)	56.3 (0.3–0.7)	1.9 (1.5–2.2)*	1.0 (0.7–1.5)*
Age	Adult	52 (24)	46.2 (0.3–0.6)	1.9 (1.5–2.5)	0.9 (0.6–1.2)
	Non-adult	41 (22)	53.7 (0.3–0.6)	2.3 (1.7–3.0)	1.2 (0.7–1.7)

The mites *P. vargasi* and *S. praecursor* have reduced host specificity, commonly occurring on several Neotropical bat species (Herrin and Tipton 1975; Moras et al. 2013; Almeida et al. 2015). The present study found *S. praecursor* specimens to strongly attach to the tragus of the host, a behavior that has been regularly recorded for this species (Fain et al. 1967; Peracchi 1990).

Our data showed a high prevalence rate of most parasites. A study with *Artibeus lituratus* and *Sturnira lilium*, frugivorous phyllostomid bats that commonly roost in treetops and/or human buildings, found much lower prevalence rates, with 3.4% and 9.1% prevalences, respectively (Dornelles and Gracioli 2017). Environmental factors and host behavior can influence prevalence rates, while type of diurnal roost occupied by the host is one of the factors that indirectly influences prevalence rates (Kunz 1982; Ter Hofstede and Fenton 2005). A host habit of changing roosts can disrupt the life cycle of ectoparasitic flies, which spend part of their life cycle inside the roosts, while such movements would not affect mites, because they spend their entire life cycle on the host's body (Kunz 1982; Lewis 1995).

The studied bat population has used Piedade cave as a diurnal roost for at least a decade and the species is known to prefer caves (Guimarães and Ferreira 2014). Caves

represent favorable diurnal roosts for many bats, as they provide a stable microclimate and protection against predators and adverse weather (Kunz 1982; Lewis 1995). Because such roosts are confined spaces, the very habit of living in a group facilitates body contact and the host-switching activity of ectoparasites. Therefore, it is plausible to hypothesize the existence of an association between the high prevalence rate recorded in the present study (94.6%) and the type of shelter (cave) used, although further investigation is needed.

The present study registered an influence of host sex on the Mean Abundance in the association between *E. clovisi* and female hosts. Higher infestation levels for females have been frequently reported in the literature (Christie et al. 2007; Patterson et al. 2008; Presley and Willig 2008). By adding the reproductive status of females to the analysis, the results were able to reveal that pregnant females were more parasitized by flies than were non-pregnant females and males. Similar results were found by Reis (2018) in a study conducted in another cave inhabited by *A. geoffroyi*. The greater fly parasitism of pregnant females is consistent with observations for other bat species such as *A. lituratus* (Bertola et al. 2005), *Megaderma lyra* (Sundari et al. 2005), *Miniopterus schreibersii* (Lourenço and Palmeirim 2008), and *Cynopterus brachyotis* (Lee et al. 2018).

The Lourenço and Palmeirim (2008) study in temperate zone showed that four parasite species had a similar reproductive pattern, reproducing more intensively during the pregnancy and nursing seasons of *M. schreibersii*, mainly on pregnant and juvenile bats. The authors concluded that this may be an adaptative trait in which the reproductive cycles of the parasite species are adjusted to the cycles of their hosts in a seasonal environment. This hypothesis could explain our results and those of other studies with similar results. Further studies focusing on the synchrony of the reproductive cycles of ectoparasites and their hosts, in other regions with marked seasonality, may bring new clarifications about this relationship.

Thus, especially for pregnant female, a lower immunological defense during the reproductive phase, due to endocrine changes inherent to reproduction (Grossman 1985; Christie et al. 2000) can facilitate their greater infestation during a period of greater reproductive activity of their ectoparasites (Lourenço and Palmeirim 2008). Reproductively active males, on the other hand, were more parasitized by *P. vargasi*, than were pregnant females. *Anoura geoffroyi* males exhibit polygyny and the mating season is relatively short (3 months) (Farias et al. 2018). The energy expenditure directed by these animals for mating in a short period may lead to a more debilitated health condition, facilitating the infestation by mites. Thus, the reproductive activity of the hosts could be an adverse factor for resistance to parasite infestations (Christie et al. 2000), even though it does not affect their body condition. However, other factors acting together can contribute to this result (Lourenço and Palmeirim 2008), needing to be tested.

Even with high parasitism rates, the data of the present study demonstrated that parasite load did not influence host BCI. Although BCI is commonly used in studies involving parasites, it has also presented contradictory results in different studies about it being correlated or not with parasite load (Marshall 1982; Christie et al. 2000; Lučan 2006; Lourenco and Palmeirim 2007; Pearce and O'Shea 2007; Lee et al.

2018). According to Postawa and Nagy (2016), even if ectoparasite density varies, the health of the host is unlikely to be affected, as ectoparasites feed mainly on host lymph and blood and do not directly consume other resources such as fatty acids, so there is no direct impact on BCI.

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